

U.S. Serial No. 09/701,132
PROVISIONAL ELECTION AND PRELIMINARY AMENDMENT
LISTING OF CLAIMS

1. (Currently Amended) An isolated and purified nucleic acid molecule which encodes all or part of an *E. coli* flagellin protein, the molecule being capable of identifying the H serotype of an *E. coli* when hybridised to a gene of the *E. coli* which encodes a flagellin protein, provided that the molecule does not encode a flagellin protein expressed by the *E. coli* H1, H7, H12 or H48 type strains.

2. (Original) A nucleic acid molecule according to claim 1 wherein the molecule is derived from a *fliC* gene.

3. (Original) A nucleic acid molecule according to claim 1 including all or part of a sequence according to any one of SEQ ID NOs:1 to 68.

4. (Original) A nucleic acid molecule according to claim 1 consisting of all or part of a sequence according to any one of SEQ ID NOs: 1 to 68.

5. (Original) A nucleic acid molecule according to claim 4 wherein the molecule is from about 10 to 20 nucleotides in length.

6. (Currently Amended) A primer selected from

<u>SEQ ID NO:</u>	<u>H</u> <u>specificity</u>	<u>Positions</u> <u>of primer 1</u>	<u>Positions</u> <u>of primer 2</u>
66	1	892-909	1172-1189
67	2	568-587	1039-1056
6,17,42	4	466-483	628-648

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7	5	697-714	877-897
8	6	565-585	799-816
9	7	553-570	1483-1500
11	9	616-633	838-855
12 (49)	10	559-579	697-717
13	11	586-606	791-810
14	12	892-909	1172-1189
15	14	586-606	793-813
16	15	640-660	817-834
68	16	649-666	925-942
18	18	589-606	802-819
17	19	607-624	538-855
20	20	574-591	760-780
21, 46	21	676-693	862-879
22	23	637-654	1336-1353
23	24	496-516	772-792
25	26	553-570	772-789
26	27	685-702	799-819
27	28	592-609	778-798
28	29	538-555	757-774
29	30	814-831	943-962
30	31	571-588	790-807
31	32	514-831	1057-1074
32	33	553-570	718-735
33	34	568-585	796-816
36, 53	38	553-573	709-729
37	39	556-573	718-735
39	41	598-615	784-801
40	42	547-567	715-735
41	43	580-597	844-861
43	45	640-657	943-963
44	46	565-582	781-801
48	49	589-609	754-771
50	51	565-582	1042-1059
51	52	598-615	829-846
54	56	697-714	877-897
10 and 38		562-579	1045-1062
24		529-549	703-723
34		769-789	1045-1065
35		520-537	715-735
47		568-585	835-852
52		988-1008	1344-1364

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the group of primers shown in the Table 3 above.

7. (Original) A method of detecting the H serotype of *E. coli* in a sample, the method comprising the following steps:

(a) contacting a gene of an *E. coli* in the sample with a nucleic acid molecule according to claim 1 in conditions sufficient to allow the nucleic acid molecule to hybridise to the gene; and

(b) detecting a nucleic acid molecule which is hybridised to the gene, to detect the H serotype of the *E. coli* in the sample.

8. (Original) A method according to claim 7 wherein the hybridised nucleic acid molecules are detected by Southern Blot analysis.

9. (Original) A method of detecting the H serotype of *E. coli* in a sample, the method comprising the following steps:

(a) contacting a gene of an *E. coli* in the sample with a pair of nucleic acid molecules according to claim 1 in conditions sufficient to allow the pair of nucleic acid molecules to hybridise to the gene; and

(b) detecting a pair of nucleic acid molecules which is hybridised to the gene, to detect the H serotype of the *E. coli* in the sample.

10. (Original) A method according to claim 9 wherein the hybridised pairs of nucleic acid molecules are detected by the polymerase chain reaction.

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11. (Original) A method for detecting the H and O serotype of *E. coli* in a sample, the method comprising the following steps:

(a) contacting a gene of the *E. coli* with a nucleic acid molecule derived from a gene encoding a transferase or a gene encoding an enzyme for the transport or processing of a polysaccharide or oligosaccharide unit, the gene being involved in the synthesis of a *E. coli* O antigen, in conditions sufficient to allow the nucleic acid molecule to hybridise to the gene;

(b) contacting a gene of an *E. coli* in the sample with a nucleic acid molecule according to claim 1 in conditions sufficient to allow the nucleic acid molecule to hybridise to the gene; and

(c) detecting nucleic acid molecules which are hybridised to the genes, to detect the H and O serotype of the *E. coli* in the sample.

12. (Currently Amended) A method according to claim 11 wherein the nucleic acid molecule of step (a) is selected from the group consisting of:

wbdH (nucleotide position 739 to 1932 of SEQ ID NO:45~~Figure 5~~),

wzx (nucleotide position 8646 to 9911 of SEQ ID NO:45~~Figure 5~~),

wzy (nucleotide position 9901 to 10953 of SEQ ID NO:45~~Figure 5~~),

wbdM (nucleotide position 11821 to 12945 of SEQ ID NO:45~~Figure 5~~),

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wbdN (nucleotide position 79 to 861 of SEQ ID
NO:56Figure-6),
wbdO (nucleotide position 2011 to 2757 of SEQ ID
NO:56Figure-6),
wbdP (nucleotide position 5257 to 6471 of SEQ ID
NO:56Figure-6),
wbdR (nucleotide position 13156 to 13821 of SEQ ID
NO:56Figure-6),
wzx (nucleotide position 2744 to 4135 of SEQ ID
NO:56Figure-6) and
wzy (nucleotide position 858 to 2042 of SEQ ID
NO:56Figure-6).

13. (Presently Amended) A method according to
claim 12 wherein the nucleic acid molecule of step (a) is a
primer selected from

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<u>Forward primer</u> <u>(base position</u> <u>of SEQ ID NO:1)</u>	<u>Reverse Primer</u> <u>(base position</u> <u>of SEQ ID NO:1)</u>
739-757	1941-1924
925-942	1731-1714
925-942	1347-1330
1165-1182	1731-1714
8646-8663	9908-9891
8906-8923	9468-9451
9150-9167	9754-9737
9976-9996	10827-10807
10113-10130	10484-10467
10931-10949	11824-11796
11821-11844	12945-12924
12042-12059	12447-12430
12258-12275	12698-12681

<u>Forward primer</u> <u>(base position</u> <u>of SEQ ID NO:2)</u>	<u>Reverse Primer</u> <u>(base position</u> <u>of SEQ ID NO:2)</u>
79-96	861-844
184-201	531-514
310-327	768-751
858-875	2042-2025
1053-1070	1619-1602
1278-1295	1913-1896
2011-2028	2757-2740
2110-2127	2493-2476
2305-2322	2682-2665
2744-2761	4135-4118
2942-2959	3628-3611
5257-5274	6471-6454
5440-5457	5973-5956
5707-5724	6231-6214
13261-13278	13629-13612
13384-13401	13731-13714

the group of primers shown in the Tables 8, ~~8A~~, 9
and ~~9A~~ above.

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14. (Original) A method according to claim 11 wherein the hybridised nucleic acid molecules are detected by Southern Blot analysis.

15. (Original) A method for detecting the H and O serotype of *E. coli* in a sample, the method comprising the following steps:

(a) contacting a gene of the *E. coli* with a pair of nucleic acid molecules derived from a gene encoding a transferase or a gene encoding an enzyme for the transport or processing of a polysaccharide or oligosaccharide unit, the gene being involved in the synthesis of a *E. coli* O antigen, in conditions sufficient to allow the pair of nucleic acid molecules to hybridise to the gene;

(b) contacting a gene of an *E. coli* in the sample with a pair of nucleic acid molecules according to claim 1 in conditions sufficient to allow the pair of nucleic acid molecules to hybridise to the gene; and

(c) detecting pairs of nucleic acid molecules which are hybridised to the genes, to detect the H and O serotype of the *E. coli* in the sample.

16. (Currently Amended) A method according to claim 15 wherein the pair of nucleic acid molecules of step (a) is selected from the group consisting of:

wbdH (nucleotide position 739 to 1932 of SEQ ID NO:45~~Figure 5~~),

wzx (nucleotide position 8646 to 9911 of SEQ ID NO:45~~Figure 5~~),

wzy (nucleotide position 9901 to 10953 of SEQ ID NO:45~~Figure 5~~),

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wbdM (nucleotide position 11821 to 12945 of SEQ ID NO:45~~Figure 5~~),

wbdN (nucleotide position 79 to 861 of SEQ ID NO:56~~Figure 6~~),

wbdO (nucleotide position 2011 to 2757 of SEQ ID NO:56~~Figure 6~~),

wbdP (nucleotide position 5257 to 6471 of SEQ ID NO:56~~Figure 6~~),

wbdR (nucleotide position 13156 to 13821 of SEQ ID NO:56~~Figure 6~~),

wzx (nucleotide position 2744 to 4135 of SEQ ID NO:56~~Figure 6~~) and

wzy (nucleotide position 858 to 2042 of SEQ ID NO:56~~Figure 6~~).

17. (Presently Amended) A method according to claim 15 wherein the nucleic acid molecules of the pair of step (a) are primers selected from

<u>Forward primer</u> <u>(base position</u> <u>of SEQ ID NO:1)</u>	<u>Reverse Primer</u> <u>(base position</u> <u>of SEQ ID NO:1)</u>
739-757	1941-1924
925-942	1731-1714
925-942	1347-1330
1165-1182	1731-1714
8646-8663	9908-9891
8906-8923	9468-9451
9150-9167	9754-9737
9976-9996	10827-10807
10113-10130	10484-10467
10931-10949	11824-11796
11821-11844	12945-12924
12042-12059	12447-12430
12258-12275	12698-12681

Forward primer	Reverse Primer
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(base position of SEQ ID NO:2)	(base position of SEQ ID NO:2)
79-96	861-844
184-201	531-514
310-327	768-751
858-875	2042-2025
1053-1070	1619-1602
1278-1295	1913-1896
2011-2028	2757-2740
2110-2127	2493-2476
2305-2322	2682-2665
2744-2761	4135-4118
2942-2959	3628-3611
5257-5274	6471-6454
5440-5457	5973-5956
5707-5724	6231-6214
13261-13278	13629-13612
13384-13401	13731-13714

the group of primers shown in the Tables ~~8, 8A, 9~~
~~and 9A~~ above.

18. (Original) A method according to claim 15 wherein the hybridised pairs of nucleic acid molecules are detected by the polymerase chain reaction.

19. (Original) A method for detecting the H and O serotype of *E. coli* in a sample, the method comprising the following steps:

(a) contacting a gene of an *E. coli* in the sample with a nucleic acid molecule according to claim 1, in conditions sufficient to allow the nucleic acid molecule to hybridise to the gene; and

(b) detecting a nucleic acid molecule which is hybridised to the gene, to detect the H and O serotype of *E. coli* in the sample.

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20. (Original) A method according to claim 19 wherein the nucleic acid molecule is according to any one of SEQ ID NOS: 9, 55, 57 to 65.

21. (Previously Amended) A method according to claim 7 wherein the sample is selected from the group consisting of a sample derived from food, a sample derived from faeces and a sample derived from a patient or animal.

22. (Previously Amended) A kit for identifying the H serotype of *E. coli*, the kit comprising at least one nucleic acid molecule according to claim 1.

23. (Currently Amended) A kit for identifying the H and O serotype of *E. coli*, the kit comprising:

(a) at least one isolated and purified nucleic acid molecule according to claim 1; and

(b) at least one nucleic acid molecule derived from and specific for a gene encoding a transferase or a gene encoding an enzyme for the transport or processing of a polysaccharide or oligosaccharide unit, the gene being involved in the synthesis of a particular *E. coli* O antigen.

24. (Currently Amended) A kit according to claim 23 wherein the at least one nucleic acid molecule of (ba) is selected from the group consisting of:

wbdH (nucleotide position 739 to 1932 of SEQ ID NO:45~~Figure 5~~),

wzx (nucleotide position 8646 to 9911 of SEQ ID NO:45~~Figure 5~~),

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wzy (nucleotide position 9901 to 10953 of SEQ ID NO:45~~Figure 5~~),
wbdM (nucleotide position 11821 to 12945 of SEQ ID NO:45~~Figure 5~~),
wbdN (nucleotide position 79 to 861 of SEQ ID NO:56~~Figure 6~~),
wbdO (nucleotide position 2011 to 2757 of SEQ ID NO:56~~Figure 6~~),
wbdP (nucleotide position 5257 to 6471 of SEQ ID NO:56~~Figure 6~~),
wbdR (nucleotide position 13156 to 13821 of SEQ ID NO:56~~Figure 6~~),
wzx (nucleotide position 2744 to 4135 of SEQ ID NO:56~~Figure 6~~) and
wzy (nucleotide position 858 to 2042 of SEQ ID NO:56~~Figure 6~~).

25. (Presently Amended) A kit according to claim 24 wherein the nucleic acid molecule of (ba) is a primer selected from

<u>Forward primer</u> <u>(base position</u> <u>of SEQ ID NO:1)</u>	<u>Reverse Primer</u> <u>(base position</u> <u>of SEQ ID NO:1)</u>
739-757	1941-1924
925-942	1731-1714
925-942	1347-1330
1165-1182	1731-1714
8646-8663	9908-9891
8906-8923	9468-9451
9150-9167	9754-9737
9976-9996	10827-10807
10113-10130	10484-10467
10931-10949	11824-11796
11821-11844	12945-12924
12042-12059	12447-12430

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12258-12275	12698-12681
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<u>Forward primer</u> <u>(base position</u> <u>of SEQ ID NO:2)</u>	<u>Reverse Primer</u> <u>(base position</u> <u>of SEQ ID NO:2)</u>
79-96	861-844
184-201	531-514
310-327	768-751
858-875	2042-2025
1053-1070	1619-1602
1278-1295	1913-1896
2011-2028	2757-2740
2110-2127	2493-2476
2305-2322	2682-2665
2744-2761	4135-4118
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5440-5457	5973-5956
5707-5724	6231-6214
13261-13278	13629-13612
13384-13401	13731-13714

the group of primers shown in the Tables 8, ~~8A~~, 9
and ~~9A~~ above.

26. (Previously Added) A method according to claim 9 wherein the sample is selected from the group consisting of a sample derived from food, a sample derived from faeces and a sample derived from a patient or animal.

27. (Previously Added) A method according to claim 11 wherein the sample is selected from the group consisting of a sample derived from food, a sample derived from faeces and a sample derived from a patient or animal.

28. (Previously Added) A method according to claim 15 wherein the sample is selected from the group consisting of a

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sample derived from food, a sample derived from faeces and a sample derived from a patient or animal.

29. (Previously Added) A method according to claim 19 wherein the sample is selected from the group consisting of a sample derived from food, a sample derived from faeces and a sample derived from a patient or animal.

30. (Previously Added) A kit for identifying the H serotype of *E. coli*, the kit comprising at least one nucleic acid molecule according to claim 6.

31. (Previously Added) A kit for identifying the H and O serotype of *E. coli*, the kit comprising:

(a) at least one nucleic acid molecule according to claim 6; and

(b) at least one nucleic acid molecule derived from and specific for a gene encoding a transferase or a gene encoding an enzyme for the transport or processing of a polysaccharide or oligosaccharide unit, the gene being involved in the synthesis of a particular *E. coli* O antigen.